IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:

Mara ROSSI et al.

Application No. 10/586,141
Filed: July 2, 2007

PROCESS FOR THE PURIFICATION OF BACTERIALLY EXPRESSED PROTEINS

Examiner: J. T. Seharasevon
Art Unit: 1646

APPEAL BRIEF

Allen C. Yun
Reg. No. 37,971
Agent for Appellants
Mara ROSSI et al.

BROWDY AND NEIMARK, P.L.L.C.
1625 K Street, N.W.
Suite 1100
Washington, D.C. 20006
Phone: 202-628-5197
Fax: 202-737-3528
Email: mail@browdyneimark.com

Attorney Docket: ROSSI10

Date: June 17, 2011

TABLE OF CONTENTS

REAL PARTY IN INTEREST

The present application is owned by Ares Trading S.A. of Aubonne, Switzerland, which is a member of Merck Serono S.A., a company under which there are a number of subsidiaries worldwide and which company is a division of Merck KGaA, headquartered in Darmstadt, Germany.

RELATED APPEALS AND INTERFERENCES

There are no related appeals or interferences.

STATUS OF CLAIMS

Claims 1-3 and 5-8 are pending in the present application and all are subject to the present appeal. Claim 4 has been cancelled.

STATUS OF AMENDMENTS

No amendment to the claims was filed subsequent to the final rejection of September 14, 2010. The claims were last amended with the amendment filed June 28, 2010, in response to a non-final Office Action and were entered by the examiner. By the Advisory Action of December 28, 2010, the examiner stated that, for purposes of appeal, the proposed amendment would be entered (even though no amendments were made to the claims subsequent to the final rejection).

SUMMARY OF CLAIMED SUBJECT MATTER

The only independent claim in this case is claim 1. No means plus function or step plus function as permitted by 35 USC §112, sixth paragraph, are present in claim 1.

Claim 1 is in Jepson format and is directed to an improvement of the process for the recovery of a chemokine protein expressed as insoluble inclusion bodies in prokaryotic host cells, where the improvement involves interposing a Reverse Phase Chromatography step between the conventional step of solubilization (e.g., denaturation with a denaturing agent) of aggregated chemokine protein in inclusion bodies and a conventional subsequent renaturation/refolding step for the solubilized/denatured protein, which is present in classical prior art processes for recovering and purifying protein from inclusion bodies.

The conventional steps for a classical prior art process for recovering and purifying a protein from inclusion bodies in prokaryotic host cells, which include the solubilization/denaturation and refolding/renaturation steps, are disclosed at page 3, lines 13-16 of the present specification. The improvement in which a Reverse Phase Chromatography (RPC) step is interposed between the solubilization/denaturation and the refolding/renaturation steps is supported in the present specification at page 2, lines 3-5; page 3, lines 4-6; and in the Example, where the step of solubilization/denaturation is disclosed at page 10,

lines 19-28, the RPC step is disclosed from page 11, line 1, to page 12, line 21, and the refolding/renaturation step is disclosed from page 12, line 23, to page 13, line 8. The interposed RPC step resulted in an increased yield of the protein.

GROUNDS OF REJECTION TO BE REVIEWED ON APPEAL

Claims 1-3 and 5-8 have been rejected under 35 U.S.C. §103(a) as being unpatentable over Musacchio et al., *Vaccine* 15(6/7):751-758 (1996) (hereinafter referred to as Musacchio) in view of Li et al., WO 98/14467 (hereinafter referred to as Li), and Proudfoot et al., WO 02/28419 (hereinafter referred to as Proudfoot). In the final Office Action of September 14, 2010, the obviousness rejection is maintained for the reasons set forth in the Office Action of March 26, 2010, where the examiner stated that:

> Therefore, it would have been obvious to one of ordinary skill in the art at the time the invention was made to modify the process of Musacchio et al. to purify chemokine mutant of SEQ ID NO:1 by the teachings of Li et al. and Proudfoot et al. with a reasonable expectation of success because Li et al. discloses the purification of chemokines from [sic] E. coli and Proudfoot discloses a mutant of SEQ ID NO: that is expressed in E. coli. The motivation to do so is provided by Proudfoot et al. in its disclosure of the process of chemokine mutant purification.

ARGUMENTS

Claims 1-3 and 5-8 Are Not Made Obvious by the Combination of Musacchio, Li and Proudfoot

For the purpose of the present obviousness rejection, all of claims 1-3 and 5-8 are considered to stand or fall together.

The examiner stated that Musacchio discloses a process for the recovery of Opc protein expressed in *E. coli* as inclusion bodies, where the recombinant protein is solubilized and purified on a reversed phase chromatography (RP-HPLC)and the eluted protein is finally refolded (abstract, page 752). The examiner acknowledged, however, that the Musacchio reference does not teach the purification of a chemokine protein using RP-HPLC.

Regarding the secondary references, the examiner took the position that Li teaches a method of purifying chemokine from inclusion bodies, where a RP-HPLC step is added to purify the protein after the renaturation step (page 24, line 20 to page 29, line 15), and not before renaturation by being interposed between solubilization and renaturation. The examiner further stated that the reference also discloses mutant chemokines (page 61). As for the Proudfoot reference, the examiner asserted that Proudfoot discloses a triple mutant of RANTES (a chemokine) polypeptide that is expressed in *E. coli* and purified. The examiner then concluded that it would

have been obvious to one of ordinary skill in the art at the time the invention was made to modify the process of Musacchio to purify the chemokine mutant of SEQ ID NO:1 by the teachings of Li and Proudfoot with a reasonable expectation of success because Li discloses the purification of chemokines from *E. coli* and Proudfoot discloses a mutant of SEQ ID NO:1 that is expressed in *E. coli*. The examiner asserted that the motivation to do so is provided by Proudfoot in its disclosure of the process of chemokine mutant purification.

The error in the examiner's rejection is that, contrary to the examiner's contention, one of ordinary skill in the art would not be motivated to interpose a Reverse Phase Chromatography (hereinafter referred to as RPC) step between a solubilizing/denaturing step and a renaturing/refolding step according to the logic used by the examiner.

As written in Jepson format, applicants concede in the preamble of claim 1 that it is conventional in the art to include the steps of solubilizing/denaturing aggregated chemokine protein in inclusion bodies and renaturing/refolding the chemokine protein in a process for the recovery and purification of a chemokine protein expressed in prokaryotic host cells as inclusion bodies. What is not conventional and not obvious is the improvement discovered by applicants when a step of Reverse Phase Chromatography (RPC) is interposed between the step of solubilizing/denaturing the protein in the inclusion body and the step of renaturing/refolding the

denatured protein. The convention in the art is to renature/refold the protein into its correct native conformation before purifying the protein using conventional protein purification techniques.

It should be pointed out that the teachings of Musacchio are a bit unusual in that purification via RPC (and followed by renaturation) was the only purification process leading to success, i.e., the recovery of pure recombinant hybrid Opc protein (see abstract and page 751, right column, where the hybrid protein is a fusion of Opc protein with the N-terminus of the high molecular weight meningococcal protein P64k as a stabilizer) renatured with a conformation sufficiently suitable to generate functional antibodies in mice capable of killing meningococci in the presence of human complement. See also page 755, first paragraph of the left column, where it is taught that "other purification procedures were used without success." One of ordinary skill in the art would readily recognize that Musacchio's interposition of an RPC step is a special unusual case where it offered the only way (after other purification procedures were used without success) to the successful recovery of a hybrid Opc protein, a protein which would also be appreciated by one of ordinary skill in the art as being produced as a hybrid fusion protein for purposes of stability.

Both the secondary Li and Proudfoot references cited and applied by the examiner teach recovering and purifying

inclusion bodies of chemokines (specifically the chemokine RANTES or a RANTES mutant protein in Proudfoot) in the conventional manner. On pages 10-11 of Proudfoot, the solubilized inclusion bodies of RANTES were renatured before proceeding with purification steps such as ion exchange chromatography. The only other mention of RANTES purification is in *Pichia pastoris,* where RANTES was expressed and secreted into the culture medium in soluble form (instead of as inclusion bodies needing to be solubilized and renatured/refolded).

Similarly, Li teaches at page 12, lines 4-6 that:

> The recovered, solubilized protein may then be purified using conventional techniques. Optionally, if deemed necessary, the target protein may be refolded prior to purification. (emphasis added)

It is therefore clear from Li's disclosures and teachings that purification steps, such as reverse phase chromatography, would be done immediately after solubilization only if there is no need for refolding; otherwise, the purification steps would be performed only after refolding. See page 15, lines 1-2, where Li teaches that:

> After solubilization and, optionally, refolding of target proteins, these proteins can be recovered and purified by methods well known in the art.

One of ordinary skill in the art can only interpret this as teaching the order of steps to be either 1) solubilization and 2) purification with no need for refolding, or 1) solubilization, 2) refolding, and 3) purification. In any event, there is absolutely no teaching in Li that would suggest a purification step, such as RPC, after solubilization but before the refolding/renaturation step, as is presently claimed. This order of steps is further confirmed by the process for purifying chemokines from bacterial inclusion bodies that is disclosed at pages 24-28 of the Li reference. Clearly, reading Li, one of ordinary skill in the art would be taught to purify chemokine only after a refolding step, if the refolding step is warranted.

Conventional wisdom is to purify only when a protein is in its native (favored) conformation, i.e., purify only after a denatured protein is renatured and refolded, as specifically taught in Li and Proudfoot. Otherwise, a solubilized but denatured protein would be a heterogeneous mixture of different (predominantly incorrect) conformations, many of which are merely transient. Purifying such a "heterogeneous" mixture, although homogeneous in molecular weight, would be problematic and contrary to conventional wisdom that would seek to obtain a homogeneous renatured protein in its native favored conformation first before starting with purification.

A person of ordinary skill in the art, armed with an understanding of the unusual circumstances in Musacchio, would certainly not be motivated to proceed against conventional wisdom as discussed above and modify the specific teachings of Li and Proudfoot regarding recovery and purification of a chemokine from inclusion bodies by interposing the RPC step of Musacchio (where the target protein is not even a chemokine and is furthermore present in the inclusion body as a fusion with a high molecular weight protein acting as a stabilizer) between the solubilizing/denaturing step and the renaturing/refolding step.

It would further be clear to one of ordinary skill in the art from reading Li's teachings of purifying chemokines from bacterial inclusion bodies that the preferred recovery and purification process taught in detail on pages 25-27 does not even include using RPC. RPC is merely disclosed in the first paragraph on page 15 of Li as being one of the many purification steps that may be used after solubilization of the chemokine inclusion bodies (and after the optional refolding step, if refolding is needed). Proudfoot's purification of RANTES from inclusion bodies does not use RPC. Thus, from a reading of Li and Proudfoot, one of ordinary skill in the art would be taught that, contrary to what is being taught in Musacchio (where purification via RPC was the only purification found to be successful for Musacchio's

hybrid fusion protein), RPC is not a critical purification step in the recovery and purification of chemokines from inclusion bodies. Accordingly, there is nothing in Li or Proudfoot to motivate one of ordinary skill in the art to even use RPC in purification since there is nothing particularly advantageous or critical about its use, much less to interpose it between the solubilizing/denaturing step and the renaturing/refolding step.

Furthermore, while Musacchio, as an isolated teaching of a special case of a hybrid fusion protein refractory to other purification procedures, discloses interposing an RPC step between the solubilization step and the refolding step for the purification of this hybrid Opc protein fusion, Musacchio also teaches that the order of the refolding and purification (i.e., RPC), at least insofar as they relate to this special case, is not important. See page 757, second full paragraph in the left column, where it is taught that, in preliminary experiments, protein renaturation carried out prior to purification leads to similar results as when pure protein (i.e., renaturation after purification) was used. Even though these results are from preliminary experiments in Musacchio, one of ordinary skill in the art would nevertheless be taught that performing the purification step prior to renaturation does not lead to any improvement. Those of ordinary skill in the art reading Musacchio would believe that the only critical parameter in Musacchio is the

use of RPC for the purification step and that this is specific to the hybrid Opc protein fusion that Musacchio was working with because RPC is certainly taught by Li and Proudfoot to not be critical in the purification of chemokines from inclusion bodies.

By contrast, the presently claimed process, which interposes an RPC step between solubilization and renaturation, leads to an improvement in achieving high/increased yield and purity of recombinant chemokines (see the present specification, page 2, lines 3-5; and page 18, last four lines, where it is disclosed that "[t]he overall recovery of the purification process estimated by RP-HPLC resulted to be over 30%, which is a very good result compared with the data in the literature").

One of ordinary skill in the art, faced with (i) the specific teachings of Li and Proudfoot that a denatured chemokine (from solubilization of chemokine inclusion bodies) should be purified only after a refolding step (and only if this refolding step is warranted in the overall recovery and purification process), (ii) the further disclosure in Musacchio that performing the RPC step before the renaturing/refolding step does not lead to any improvement, and (iii) the teachings in Li and Proudfoot that RPC is not critical in the purification of chemokines, would have absolutely no motivation to act against conventional wisdom and interpose an RPC purification step between

solubilization/denaturation and renaturation/refolding for the recovery and purification of a chemokine from inclusion bodies. There is no suggestion anywhere of any reason to modify the procedure of Li or Proudfoot. Thus, not only would this person of ordinary skill in the art have no such motivation but this same person would indeed also have no expectation that such an interposition contrary to conventional wisdom would lead to an improvement in the yield of purified protein.

CONCLUSION

None of the present claims are rendered obvious by the prior art references of record. For all of the reasons herein, reversal of the examiner and allowance of all of the claims now present in the case are earnestly solicited.

Respectfully submitted,

BROWDY AND NEIMARK, P.L.L.C.
Attorneys for Applicant(s)

By /ACY/
Allen C. Yun
Registration No. 37,971

ACY:pp

CLAIMS APPENDIX

This listing of claims includes all of the claims involved in the appeal.

Listing of Claims:

1. In a process for the recovery of a chemokine expressed in prokaryotic host cells as inclusion bodies and its subsequent purification, comprising a step of solubilization of aggregated chemokine protein in inclusion bodies/denaturation and a step of renaturation/refolding, the improvement consisting of interposing a Reverse Phase Chromatography step between the step of solubilization of the aggregated proteins in the inclusion bodies/denaturation and the renaturation/refolding step.

2. The process of claim 1, in which the prokaryotic cells are bacterial cells.

3. The process of claim 2, in which the bacterial cells are *Escherichia coli* cells.

5. The process of claim 1, wherein the chemokine is the mutant protein having the sequence of SEQ ID NO:1.

6. The process of claim 1, characterized in that the following steps are performed:

a) solubilizing the aggregated chemokine proteins in the inclusion bodies;

b) subjecting the solubilized chemokine proteins to Reverse Phase Chromatography;

c) subjecting the product obtained from step b) to a renaturation/refolding step; and

d) subjecting the product obtained from step c) to a chromatographic step selected from size exclusion chromatography, ion exchange chromatography, affinity chromatography.

7. The process of claim 1, characterized in that the following steps are performed:

a) solubilizing the aggregated chemokine proteins in the inclusion bodies;

b) subjecting the solubilized chemokine protein to Reverse Phase Chromatography;

c) subjecting the product obtained from step b) to a renaturation/refolding step; and

d) subjecting the product obtained from step c) to two ion exchange chromatography steps.

8. The process of claim 1, wherein, after the solubilizing and/or refolding step, a filtration step is performed.

EVIDENCE APPENDIX

None

RELATED PROCEEDINGS APPENDIX

None